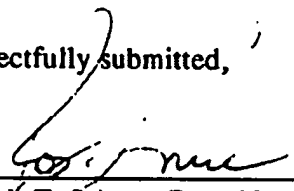


Applicant respectfully submits that the application is now in condition for allowance.
Early notification of such action is earnestly solicited.

Respectfully submitted,


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Appln. Serial No.: 09/431,451

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Filing Date: November 1, 1999

Examiner: Sisson, B.

Applicant: Senapathy, P.

Attorney Docket No.: 34623.005

TECH CENTER 1600/290

Title: METHOD FOR AMPLIFYING SEQUENCES FROM UNKNOWN DNA

"MARKED UP" CLAIMS AS AMENDED, 37 CFR §1.121(c)(1)(ii)

1. **[AMENDED FOUR TIMES]** A method of amplifying desired regions of nucleic acid from a nucleic acid template comprising:
 - (a) providing a plurality of first PCR primers, each first primer having an overall length of **[at least] from** about 10 nucleotides **to about 30 nucleotides** and further having a region of fixed nucleotide sequence identical or complementary to a consensus sequence of interest and a region of randomized nucleotide sequence located 5' to, 3' to, or flanking the region of fixed nucleotide sequence;
 - (b) providing a plurality of second PCR primers, each second primer having an overall length of **[at least] from** about 10 nucleotides **to about 30 nucleotides** and further having a region of arbitrary, yet fixed nucleotide sequence and a region of randomized nucleotide sequence located 5' to, 3' to, or flanking the region of fixed nucleotide sequence; and then
 - (c) amplifying the nucleic acid template via the PCR using the plurality of first PCR primers and the plurality of second PCR primers under conditions wherein a subset of the plurality first primers binds to the consensus sequence of interest substantially wherever it occurs in the template, and a subset of the plurality of second primers binds to the template at locations removed from the first primers such that nucleic acid regions flanked by the first primer and the second primer are specifically amplified.

12. **[AMENDED FOUR TIMES]** A method of amplifying exons from a nucleic acid template comprising:
 - (a) providing a plurality of first PCR primers, each first primer having an overall length of **[at least] from** about 10 nucleotides **to about 30 nucleotides** and further having a region of fixed nucleotide sequence identical or complementary to a consensus sequence of a 3' splice region and a region of randomized nucleotide sequence located 5' to, 3' to, or flanking the region of fixed nucleotide sequence;

- (b) providing a plurality of second PCR primers, each second primer having an overall length of [at least] from about 10 nucleotides to about 30 nucleotides and further having a region of fixed nucleotide sequence reversely complementary to a consensus sequence of a 5' splice region and a region of randomized nucleotide sequence located 5' to, 3' to, or flanking the region of fixed nucleotide sequence; and then
- (c) amplifying the nucleic acid template via the PCR using the plurality of first PCR primers and the plurality of second PCR primers under conditions wherein a subset of the plurality first primers binds to a sequence reversely complementary to the 3' splice consensus sequence substantially wherever it occurs in the template, and a subset of the plurality of second primers binds to the 5' splice consensus sequence substantially wherever it occurs in the template, such that exons flanked by the first primer and the second primer are specifically amplified.

19. **[AMENDED FOUR TIMES]** A method of amplifying regions flanking a consensus sequence in a nucleic acid template of totally or partially unknown sequence comprising:

- (a) providing a plurality of first PCR primers, each first primer having an overall length of [at least] from about 10 nucleotides to about 30 nucleotides and further having a region of fixed nucleotide sequence identical or complementary to a consensus sequence of interest and a region of randomized nucleotide sequence located 5' to, 3' to, or flanking the region of fixed nucleotide sequence;
- (b) providing a plurality of second PCR primers, each second primer having an overall length of [at least] from about 10 nucleotides to about 30 nucleotides and further having a region of arbitrary, yet fixed nucleotide sequence and a region of randomized nucleotide sequence located 5' to, 3' to, or flanking the region of fixed nucleotide sequence; then
- (c) amplifying the nucleic acid template via the PCR using the plurality of first PCR primers and the plurality of second PCR primers under conditions wherein a subset of the plurality first primers binds to the consensus sequence of interest substantially wherever it occurs in the template, and a subset of the plurality of second primers binds to the template at locations removed from the first primers such that nucleic acid regions flanked by the first primer and the second primer are specifically amplified; then
- (d) incorporating the amplified nucleic acid of step (c) into a library;
- (e) sequencing a portion of amplified nucleic acid from a particular clone from the library of step (d) and providing a third PCR primer of unique sequence and having an overall length of at least about 10 nucleotides which will prime PCR amplification from the sequenced portion of DNA;
- (f) providing a plurality of fourth PCR primers, each fourth primer having an overall length of at least about 10 nucleotides and further having a region of

- arbitrary, yet fixed nucleotide sequence and a region of randomized nucleotide sequence located 5' to, 3' to, or flanking the region of fixed nucleotide sequence; and then
- (g) amplifying the nucleic acid present in the template via the PCR using the third PCR primer and the plurality of fourth PCR primers under conditions wherein the third primer binds to the sequenced portion of nucleic acid from step (e), and a subset of the plurality of fourth primers binds to the template at locations removed from the third primers such that nucleic acid regions flanked by the third primer and the fourth primer are specifically amplified.